ISSN 1022-7954, Russian Journal of Genetics, 2007, Vol. 43, No. 12, pp. 1413–1414. © Pleiades Publishing, Inc., 2007.
Original Russian Text © B.R. Kuluev, A.V. Chemeris, 2007, published in Genetika, 2007, Vol. 43, No. 12, pp. 1682–1684.

SHORT COMMUNICATIONS

Amplification and Cloning of Dahlia Mosaic Virus and Carnation Etched Ring Virus Promoters

B. R. Kuluev[a] and A. V. Chemeris[b]

[a] *Department of Biochemistry and Biotechnology, Bashkir State University, Ufa, 450074 Russia; e-mail: Kuluev@bk.ru*
[b] *Ufa Research Center, Institute of Biochemistry and Genetics, Russian Academy of Sciences, Ufa, 450054 Russia*

Received November 10, 2006; in final form, May 17, 2007

Abstract—Amplification and cloning of dahlia mosaic virus promoter were carried out for the first time. Sequence analysis showed homology between this promoter and the promoters of other caulimoviruses. In addition, amplification and cloning of the carnation etched ring virus promoter was performed.

DOI: 10.1134/S1022795407120113

In transgenic plants, substantial levels of the alien gene expression is often reached through utilization of strong constitutive promoters of caulimoviruses instead of the transgenic promoters. The most comprehensively studied promoter is the 35S promoter of cauliflower mosaic virus (CMV) [1]. This promoter was found to be stronger than the mannopine synthase and octopine synthase promoters, widely used earlier in plant genetic engineering. In some cases, however, the CMV 35S promoter appeared to be not strong enough. In this context, a search for the new stronger promoters deserves special interest [2]. At the same time, the CMV 35S promoter in many respects was shown to be universal. Because of this, a search for analogous promoters was performed among other caulimoviruses. As far as dahlia and carnation are infected by caulimoviruses, the plants with typical symptoms of viral diseases were first selected. Promoter of the carnation etched ring virus was amplified from the leaves of different carnation species, received from the Institute of Virology, Microbiology, and Biological Safety (Braunschweig, Germany). Dahlia plants with typically mosaic leaves were found in Ufa and used in the analysis. Molecular biological analysis showed that the leaves were infected with dahlia mosaic virus (DMV).

Amplification and cloning of dahlia mosaic virus promoter. Dalia mosaic virus causes the formation of light spots in the vein area, and it naturally affects only the members of the genus *Dahlia*. The DMC genome is only partly sequenced. The GenBank database contains the sequences of few individual genes (EF203678, AY291585, and AY971810). Neither promoter, nor the promoter region is sequenced, making impossible the design of primers suitable for amplification of the DMV promoter. For these reasons, design of amplification primers was performed using known DMV genomic sequences, and the sequences most close to the promoter were selected. Primers 5'-CAGTGACTCATCCTCCAATA-3' and 5'-AATATCTACTGACATTTCTT-3' were found to be most suitable. According to theoretical calculations, the size of the amplified fragment should be about 1300 bp. Amplification was conducted at an annealing temperature of 48°C. The reaction mixture contained dimethyl sulfoxide (DMSO) in a final concentration of 1%. The size of the amplified fragment was about 1300 bp. This DNA fragment was cloned into phagemid vector pKRX and sequenced (EF463101). Sequence analysis showed that the cloned fragment contained a part of the gene 6, promoter, large intergenic spacer, and open reading frame 7 of dahlia mosaic virus. Alignment of the promoter sequence determined with the dahlia mosaic virus gene 6 sequence from the GenBank using the MEGALIGN program from the LASERGENE software package showed their concordance. It can be thus concluded that the present study presents the first sequencing and primary analysis of the promoter of dahlia mosaic virus. It was demonstrated that the DMV promoter was homologous to the promoters of other caulimoviruses. For instance, the DMV promoter was 60% homologous to the promoter of the figwort mosaic virus [3] and 63% homologous to the promoter of the mirabilis mosaic virus [4]. Comparative analysis of the promoter regulatory regions from the DMV, figwort mosaic virus, and mirabilis mosaic virus showed the following. The CCACT box in the figwort mosaic virus had canonic sequence, which in DMV was represented by the CCAAT sequence, and by the GCAAC sequence in the mirabilis mosaic virus. In the mosaic viruses of figwort and mirabilis, the TGACG box was represented by the TGACG sequence, while in DMV, T was replaced with A. The TATA box in all three species was indistinguishable and represented by the TATATAA canonic sequence. An analysis performed provided localization of the promoter, as well as primer selection for amplification of the promoter itself. Judging by the homology to relative caulimoviruses, the promoter size could not be larger than 400 bp. Finally, the 442-bp promoter seg-

ment of dahlia mosaic virus was amplified and cloned. This sequence was submitted to the GenBank under the accession number EF513491. The promoter analyzed was also cloned into the binary vector pCambia 1304 [http://www.cambia.org.au], within which it controlled the expression of the reporter genes *uidA* and *gfp*. Activity of the DMV promoter was demonstrated in the cells of *Escherichia coli* and *Agrobacterium tumefaciens*, as well as in the protoplasts of tobacco.

Amplification and cloning of the carnation etched ring virus promoter. The GenBank database contains complete genomic sequence of the carnation etched ring virus. Using multiple alignment with different caulimoviruses, it was demonstrated that this virus is closer to the CMV, than to other viruses. For this reason, to evaluate the promoter position, the CERV genome was aligned with the genomes of four CMV strains. Next, primers for amplification of the CERV promoter were selected, taking into consideration alignment with all caulimoviruses. In this case, more conservative regions were preferred. Three primers were selected: CERVFltF1, 5'-ATTAAAAGAA-CAATGGCAAG-3', CERVFltF2, 5'-AGAAGATT-TAATGGCAATCC-3', and CERVFltR, 5'-AGGACT-TAGGCTCAACACAT-3'. The expected amplicon size in case of amplification with primers CERVFltF1 and CERVFltR was 651 bp, while in the reaction with primers CERVFltF2 and CERVFltR it was 501 bp. Using four carnation leaf samples, we managed to amplify the DNA fragments of 500 and 650 bp. The analysis performed demonstrated the presence of CERV in the carnation leaves. Both DNA fragments, which differed only in the primer positions, were cloned into phagemid vectors pKRX and pBluescriptII KS (±) and sequenced. The 455-bp promoter fragment was submitted to the GenBank under the accession number EF513492. The search for similar sequences in the gene database was performed with the help of the MEGA-BLAST software program, available at the NCBI website, and showed 95% of homology between the promoter fragment sequenced and the promoter of the CERV isolate from the United States. Multiple alignments of the caulimovirus promoters showed that the CERV promoter is most close to the CMV 35S promoter. Comparative sequence analysis of the promoters from cauliflower mosaic virus and carnation etched ring virus showed that CCACT box in the CERV promoter differed from that in the CMV 35S promoter and was represented by the CTACT sequence. The TATA box was represented by the TATATAA sequence, while TGACG box, similarly to the CMV 35S promoter, was represented by the TGACG canonic sequence. Alignment of the CERV promoter with the promoters of other caulimoviruses made it possible to determine the exact positions of the main domains, as well as of the borders of the promoter itself. The CERV promoter starts at the nucleotide position 6750 and ends at the nucleotide position 7102. Thus, the promoter size is 352 bp. It can be hypothesized that this size is optimal for the highest promoter activity. To evaluate activity of this promoter, using binary vectors of the pCambia family, plasmids were constructed, where the CERV promoter controlled expression of the *gfp* and *uidA* reporter genes. Activity of the CERV promoter was demonstrated in the cells of *Escherichia coli* and *Agrobacterium tumefaciens*, as well as in the protoplasts of tobacco.

REFERENCES

1. Odell, J.T., Nagy, F., and Chua, N.H., Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter, *Nature*, 1985, vol. 313, no. 6005, pp. 810–812.
2. Mitsuhara, I., Ugaki, M., Hirochika, H., et al., Efficient Promoter Cassettes for Enhanced Expression of Foreign Genes in Dicotyledonous and Monocotyledonous Plants, *Plant Cell Physiol.*, 1996, vol. 37, no. 1, pp. 49–59.
3. Richins, R.D., Scholthof, H.B., and Shepherd, R.J., Sequence of Figwort Mosaic Virus DNA, *Nucleic Acids Res.,* 1987, vol. 15, no. 20, pp. 8451–8465.
4. Dey, N. and Maiti, I.B., Structure and Promoter/Leader Deletion Analysis of Mirabilis Mosaic Virus (MMV) Full-Length Transcript Promoter in Transgenic Plants, *Plant. Mol. Biol.*, 1999, vol. 40, no. 5, pp. 771–782.